

Listing of Claims:

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C/ Claims 1-11 (Canceled)

1                   12.   (Previously Amended) A commercial-scale method of sialylating a  
2    saccharide group on a recombinant glycoprotein, the method comprising contacting a saccharide  
3    group which comprises a galactose or N-acetylgalactosamine acceptor moiety on a recombinant  
4    glycoprotein with a sialic acid donor moiety and a recombinant bacterial sialyltransferase in a  
5    reaction mixture which provides reactants required for sialyltransferase activity for a sufficient  
6    time and under appropriate conditions to transfer sialic acid from said sialic acid donor moiety to  
7    said saccharide group.

1                   13.   (Original) The method of claim 12, wherein the bacterial sialyltransferase  
2    has an amino acid sequence which is at least 50% identical to an amino acid sequence of a  
3    *Neisseria meningitidis* 2,3-sialyltransferase.

1                   14.   (Original) The method of claim 13, wherein the bacterial sialyltransferase is  
2    a *Neisseria meningitidis* 2,3-sialyltransferase.

1                   15.   (Original) The method of claim 12, wherein the bacterial sialyltransferase  
2    has an amino acid sequence which is at least 50% identical to an amino acid sequence of a  
3    *Photobacterium damsela* 2,6-sialyltransferase.

1                   16.   (Original) The method of claim 15, wherein the bacterial sialyltransferase is  
2    a *Photobacterium damsela* 2,6-sialyltransferase.

1                   17.   (Original) The method of claim 12, wherein the bacterial sialyltransferase  
2    has an amino acid sequence which is at least 50% identical to an amino acid sequence of a  
3    *Haemophilus* 2,3-sialyltransferase.

18. (Original) The method of claim 17, wherein the sialyltransferase is a  
*Haemophilus* 2,3-sialyltransferase.

19. (Original) The method of claim 12, wherein the bacterial sialyltransferase  
has an amino acid sequence which is at least 50% identical to an amino acid sequence of a  
*Campylobacter jejuni* 2,3-sialyltransferase.

20. (Original) The method of claim 19, wherein the sialyltransferase is a  
*Campylobacter jejuni* 2,3-sialyltransferase.

21-22. (Cancelled)

23. (Previously Amended) A commercial-scale method of sialylating a  
saccharide group on a recombinant glycoprotein, the method comprising contacting a saccharide  
group which comprises a galactose or an N-acetylgalactosamine acceptor moiety on a  
recombinant glycoprotein with a sialic acid donor moiety and a bacterial sialyltransferase in a  
reaction mixture which provides reactants required for sialyltransferase activity for a sufficient  
time and under appropriate conditions to transfer sialic acid from said sialic acid donor moiety to  
said saccharide.

24. (Original) The method of claim 23, wherein the bacterial sialyltransferase  
has an amino acid sequence which is at least 50% identical to an amino acid sequence of a  
*Photobacterium damsela* 2,6-sialyltransferase.

25. (Original) The method of claim 24, wherein the bacterial sialyltransferase is  
a *Photobacterium damsela* 2,6-sialyltransferase.

26. (Original) The method of claim 22, wherein the bacterial sialyltransferase has an amino acid sequence which is at least 50% identical to an amino acid sequence of a *Neisseria meningitidis* 2,3-sialyltransferase.

27. (Original) The method of claim 26, wherein the sialyltransferase is a *Neisseria meningitidis* 2,3-sialyltransferase.

28. (Original) The method of claim 23, wherein the bacterial sialyltransferase has an amino acid sequence which is at least 50% identical to an amino acid sequence of a *Campylobacter jejuni* 2,3-sialyltransferase.

29. (Original) The method of claim 28, wherein the sialyltransferase is a *Campylobacter jejuni* 2,3-sialyltransferase.

30. (Original) The method of claim 23, wherein the bacterial sialyltransferase has an amino acid sequence which is at least 50% identical to an amino acid sequence of a *Haemophilus* 2,3-sialyltransferase.

31. (Original) The method of claim 30, wherein the sialyltransferase is a *Haemophilus* 2,3-sialyltransferase.

32-43. (Canceled)

44. (Previously amended) A commercial-scale method for *in vitro* sialylation of saccharide groups on a glycoprotein, said method comprising contacting said saccharide groups with a sialyltransferase, wherein the sialyltransferase is a bacterial sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase activity for a sufficient time and

5 under appropriate conditions to transfer sialic acid from said sialic acid donor moiety to said  
6 saccharide group.

1 45. (Original) The method of claim 44, wherein the bacterial sialyltransferase is  
2 a recombinant sialyltransferase.

1 46. (Original) The method of claim 44, wherein the bacterial sialyltransferase  
2 has an amino acid sequence which is at least 50% identical to an amino acid sequence of a  
3 *Neisseria meningitidis* 2,3-sialyltransferase.

1 47. (Original) The method of claim 46, wherein the bacterial sialyltransferase is  
2 a *Neisseria meningitidis* 2,3-sialyltransferase.

1 48. (Original) The method of claim 44, wherein the bacterial sialyltransferase  
2 has an amino acid sequence which is at least 50% identical to an amino acid sequence of a  
3 *Photobacterium damsela* 2,6-sialyltransferase.

1 49. (Original) The method of claim 48, wherein the bacterial sialyltransferase is  
2 a *Photobacterium damsela* 2,6-sialyltransferase.

1 50. (Original) The method of claim 44, wherein the bacterial sialyltransferase  
2 has an amino acid sequence which is at least 50% identical to an amino acid sequence of a  
3 *Campylobacter jejuni* 2,3-sialyltransferase.

1 51. (Original) The method of claim 50, wherein the sialyltransferase is a  
2 *Campylobacter jejuni* 2,3-sialyltransferase.

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1                    52. (Original) The method of claim 44, wherein the bacterial sialyltransferase  
2 has an amino acid sequence which is at least 50% identical to an amino acid sequence of a  
3 *Haemophilus* 2,3-sialyltransferase.

1                    53. (Original) The method of claim 52, wherein the sialyltransferase is a  
2 *Haemophilus* 2,3-sialyltransferase.

1                    54. (Canceled)

1                    55. (Original) The method of claim 54, wherein the CMP-sialic acid is  
2 enzymatically generated *in situ*.

1                    56. (Original) The method of claim 32, wherein the sialic acid is selected from  
2 the group consisting of NeuAc and NeuGc.

1                    57. (Previously amended) A commercial-scale method for *in vitro* sialylation of  
2 terminal galactose residues on a glycoprotein, said method comprising contacting said  
3 glycoprotein with a reaction mixture that comprises a sialyltransferase, a sialic acid donor  
4 moiety, and other reactants required for sialyltransferase activity, for a sufficient time and under  
5 appropriate conditions to transfer sialic acid from said sialic acid donor moiety to said terminal  
6 galactose residues.

1                    58. (Original) The method of claim 57, wherein the method further comprises  
2 contacting the saccharide groups with an ST6GalII sialyltransferase.

1                    59. (Previously added) A method for *in vitro* sialylation of terminal galactose  
2 residues present on a glycoprotein, said method comprising contacting said glycoprotein with a  
3 reaction mixture that comprises a sialyltransferase, wherein the sialyltransferase is a bacterial

4 sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase  
5 activity, for a sufficient time and under appropriate conditions to transfer sialic acid from said  
6 sialic acid donor moiety to said terminal galactose residues, wherein a greater percentage of  
7 terminal galactose residues are sialylated compared to an unaltered glycoprotein.

1 60. (Previously added) The method of claim 59, wherein at least 80% of the  
2 terminal galactose residues present on the glycoprotein are sialylated.

1 61. (Previously added) The method of claim 60, wherein at least 90% of the  
2 terminal galactose residues present on the glycoprotein are sialylated.

1 62. (Previously added) The method of claim 59, wherein the terminal galactose  
2 residues comprise one or more saccharides selected from the group consisting of  
3 Gal $\beta$ 1,4GlcNAc, Gal $\beta$ 1,4GalNAc, Gal $\beta$ 1,3GalNAc, Gal $\beta$ 1,3GlcNAc, Gal $\beta$ 1,3Ara,  
4 Gal $\beta$ 1,6GlcNAc, and Gal $\beta$ 1,4Glc.

1 63. (Previously added) The method of claim 62, wherein the terminal galactose  
2 residues comprise Gal $\beta$ 1,4GlcNAc or Gal $\beta$ 1,3GlcNAc.

1 64. (Previously added) The method of claim 63, wherein at least 80% of the  
2 terminal Gal $\beta$ 1,4GlcNAc residues present on the glycoprotein are sialylated.

1 65. (Previously added) The method of claim 63, wherein at least 80% of the  
2 terminal Gal $\beta$ 1,3GlcNAc residues present on the glycoprotein are sialylated.

1 66. (Previously added) The method of claim 59, wherein the terminal galactose  
2 residues are present on an O-linked oligosaccharide.

1 67. (Previously added) The method of claim 59, wherein the terminal galactose  
2 residues are present on an N-linked oligosaccharide.

68. (Previously added) The method of claim 59, wherein the sialyltransferase includes a sialyl motif which has an amino acid sequence that is at least about 40% identical to a sialyl motif from a sialyltransferase selected from the group consisting of ST3Gal I, ST6Gal I, and ST3Gal III.

69. (Previously added) The method of claim 68, wherein the sialyltransferase is an ST3Gal III.

70. (Previously added) The method of claim 69, wherein the sialyltransferase is a rat ST3Gal III.

71. (Previously added) The method of claim 68, wherein the sialyltransferase is an ST3Gal IV.

72. (Previously added) The method of claim 68, wherein the sialyltransferase is an ST6Gal I.

73. (Previously added) The method of claim 68, wherein the sialyltransferase is an ST3Gal I.

74. (Previously added) The method of claim 59, wherein the bacterial sialyltransferase has an amino acid sequence which is at least 50% identical to an amino acid sequence of a *Neisseria meningitidis* 2,3-sialyltransferase.

75. (Previously added) The method of claim 74, wherein the bacterial sialyltransferase is a *Neisseria meningitidis* 2,3-sialyltransferase.

76. (Previously added) The method of claim 73, wherein the bacterial sialyltransferase has an amino acid sequence which is at least 50% identical to an amino acid sequence of a *Photobacterium damsela* 2,6-sialyltransferase.

1 77. (Previously added) The method of claim 76, wherein the bacterial  
2 sialyltransferase is a *Photobacterium damsela* 2,6-sialyltransferase.

1 78. (Previously added) The method of claim 59, wherein the bacterial  
2 sialyltransferase has an amino acid sequence which is at least 50% identical to an amino acid  
3 sequence of a *Haemophilus* 2,3-sialyltransferase.

1 79. (Previously added ) The method of claim 78, wherein the sialyltransferase  
2 is a *Haemophilus* 2,3-sialyltransferase.

1 80. (Previously added ) The method of claim 59, wherein the bacterial  
2 sialyltransferase has an amino acid sequence which is at least 50% identical to an amino acid  
3 sequence of a *Campylobacter jejuni* 2,3-sialyltransferase.

1 81. (Previously added ) The method of claim 80, wherein the sialyltransferase  
2 is a *Campylobacter jejuni* 2,3-sialyltransferase.

1 82. (Previously amended) A commercial-scale method for altering the  
2 glycosylation pattern of a glycoprotein *in vitro*, the method comprising contacting a  
3 glycoprotein-linked saccharide with a galactosyltransferase in the presence of UDP-galactose  
4 under suitable conditions for the galactosyltransferase to transfer a galactose residue from the  
5 UDP-galactose to the saccharide to form a galactosylated saccharide.

83-97. (Canceled)

1 98. (Previously added ) The method of claim 12, wherein the glycoprotein  
2 comprises an immunoglobulin.

1 99. (Previously added ) The method of claim 23, wherein the glycoprotein  
2 comprises an immunoglobulin.



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1 100. (Previously added ) The method of claim 44, wherein the glycoprotein  
2 comprises an immunoglobulin.

1 101. (Previously added ) The method of claim 57, wherein the glycoprotein  
2 comprises an immunoglobulin.

1 102. (Previously added ) The method of claim 82, wherein the glycoprotein  
2 comprises an immunoglobulin.

1 103. (New) A method for *in vitro* sialylation of a saccharide group present on a  
2 glycoprotein, said method comprising:  
3 (a) modifying said glycoprotein to create an acceptor; and  
4 (b) sialylating said acceptor formed in (a) with a sialyltransferase in the presence  
5 of a CMP derivative of a sialic acid using an  $\alpha(2,3)$ sialyltransferase under conditions in which  
6 sialic acid is transferred to a non-reducing sugar present on said glycoprotein.

1 104. (New) The method according to claim 103, wherein said modifying  
2 comprises:

3 galactosylating a compound of the formula  $\text{GlcNR}'\beta(1\rightarrow 3)\text{Gal}\beta\text{-OR}$  with a  
4 galactosyltransferase in the presence of a UDP-galactose under conditions sufficient to form  
5  $\text{Gal}\beta(1\rightarrow 4)\text{GlcNR}'\beta(1\rightarrow 3)\text{Gal}\beta\text{-OR}$ , wherein:

6 R is a member selected from the group consisting of an amino acid, a saccharide,  
7 an oligosaccharide, and an aglycon group having at least one carbon; and wherein:

8 R' is a member selected from the group consisting of acetyl and allyloxycarbonyl.

1 105. (New) The method according to claim 104, wherein R is linked to or is  
2 part of a glycoprotein.

106. (New) The method according to claim 104, wherein said galactosylating and sialylating are carried out enzymatically.

107. (New) The method according to claim 104, wherein said galactosylating is carried out as part of a galactosyltransferase cycle.

108. (New) The method according to claim 104, wherein said sialylating is carried out as part of a sialyltransferase cycle.

109. (New) The method according to claim 104, wherein said method comprises carrying out said galactosylating and said sialylating in a single reaction mixture that contains both a sialyltransferase and a galactosyltransferase.

110. (New) The method according to claim 109, wherein said sialyltransferase, said galactosyltransferase, and said GlcNR' $\beta$ (1 $\rightarrow$ 3)Gal $\beta$ -OR are combined in an initial reaction mixture.

111. (New) The method according to claim 109, wherein said method further comprises the addition of said sialyltransferase, said galactosyltransferase, and said GlcNR' $\beta$ (1 $\rightarrow$ 3)Gal $\beta$ -OR for a second glycosyltransferase cycle once a first glycosyltransferase cycle has neared completion.

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